

THE INTERRELATIONS AMONG THREE HORMONAL-CONTROLLED CHARACTERS IN THE ADULT FEMALE SHORE CRAB, *CARCINUS MAENAS* (L.)

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The three decapod characters, molting, ovarian developing stages and shell color changes, have all been shown to be under hormonal control (Brown and Jones, 1947; Carlisle, 1953a, 1953b; Démeusy, 1962; Echaliér, 1955, 1956; Lenel and Veillet, 1951; and Passano, 1951b, etc.).

While much experimental work has been done by the above authors on this field, so far no attempt has yet been made to investigate the interrelation among these characters under natural conditions. This study was therefore carried out in the Department of Zoology, University of Glasgow by examining specimens of female *Carcinus maenas* (L.) collected from Millport, Isle of Cumbrae between November, 1961, and March, 1964.

MATERIALS AND METHODS

The crabs were taken in baited creels off the Millport Marine Laboratory near Old Pier at a depth of 3 to 4 meters. At least one lot of crabs was taken nearly every month so that the study included examples in all seasons. Crabs with internal or external *Sacculina* were discarded, since the effect of parasitic castration would retard normal ovarian growth (Charniaux-Cotton, 1960, p. 419), inhibit molting (Passano, 1960, p. 483) as well as absorb carotenoid pigments (Lenel, 1954).

All the crabs were adults with carapace width greater than 40 mm. The number of female crabs included in this study was 519, but the conclusions reached are found to be statistically significant in nearly all cases.

Classification of the stages of the ovary was made according to the following scheme:

White. The ovary was redeveloping after the spent stage. In section, active proliferation was visible. Resorption of yolk and degeneration of old oocytes from the last spawning were completed. In many cases the crab could be recognized as having been recently fertilized after the last molt, the spermathecae appearing swollen.

Yellow. A stage subsequent to white. The yellow colors are due to yolk deposition. The ovary had enlarged considerably but was far from its full size.

Orange. At this stage yolk deposition had gone on still further so that the ovary acquired its orange color. The ovary had enlarged and it was during this stage that it reached full size and was ready for egg-laying.

Spent. This included crabs that had spawned recently, but mostly had not yet undergone a molt since. All berried crabs were in this category. The ovary was here reduced in size, with still a few mature oocytes.

Classification of the molting stages followed that of Passano (1960, p. 477) which is a modified scheme of Drach's (1939). Since this classification is well known, a reiteration here is not necessary. Because of the fact that the crabs were collected in baited creels the non-feeding stages D_3 , D_4 (later premolts), E (molting) as well as A_1 , A_2 and B_1 (early postmolt) were missing from the sample throughout the study. Therefore, the above detailed classification had to be modified in a convenient way for this particular work. Accordingly, the crabs were divided into (a) postmolt, which included stages from B_2 to C_3 , (b) intermolt, including only C_4 , and (c) premolt, which consisted of D_0 to D_2 crabs. The above-mentioned missing stages around molting should be very short (Passano, 1960) in the molting cycle so that conclusion in this work would not be affected.

The color stages were classified on the basis of the color on the ventral surface of the animal excluding the carapace, but including the abdomen in its natural position. These were green, yellow and orange-red; occasionally blue and brown colors were found present with the above. The latter two colors, especially blue, when present usually occupied much smaller areas than the first three, which might be present on the whole of the ventral side. In cases where two colors were found the crab was recorded as belonging half to one and half to another color; similarly, when three colors occurred in the same crab it was recorded as one-third belonging to each of the three colors, irrespective of the proportion in areas each color occupied. The numbers of crabs recorded in Tables III and V were taken to the nearest halves. As the blue and brown colors occurred but rarely (Table III), they are not represented in Figure 2.

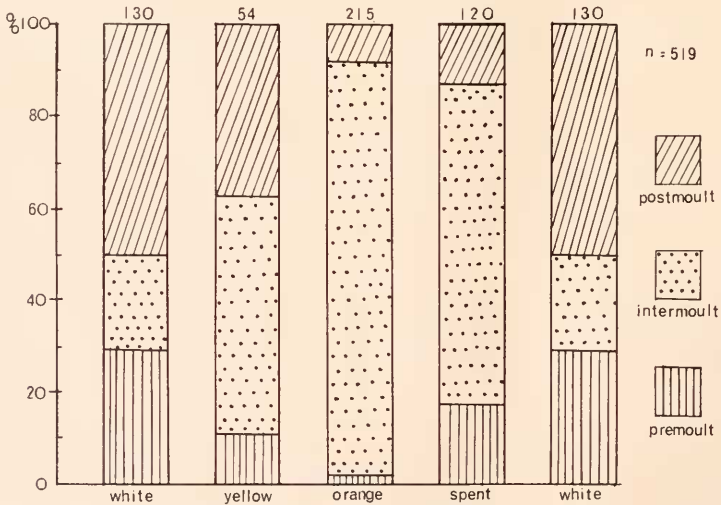


FIGURE 1. Relation between various ovarian and molting stages. Abscissa: different stages of the ovaries (see text). Postmolt = stages B_2 - C_3 ; intermolt = stage C_4 ; premolt = stages D_0 - D_2 .

TABLE I

Relation between various ovarian and molting stages. Postmolt = stages B₂-C₃; intermolt = stage C₄; Premolt = stages D₀-D₂. Percent in bracket.

	Ovary				Total
	White	Yellow	Orange	Spent	
Postmolt	65 (50.0)	20 (37.0)	17 (7.9)	15 (12.5)	117
Intermolt	27 (20.7)	28 (51.8)	194 (90.2)	84 (70.0)	333
Premolt	38 (29.2)	6 (11.1)	4 (1.9)	21 (17.5)	69
Total	130 (100)	54 (100)	215 (100)	120 (100)	519

These colors were apparently due to pigments in the cuticular layers in the integument of the animals. The biochemical nature of the changes of coloration in the *Carcinus* shell has been worked out in some detail by Lenel (1953, 1954, 1955) and Lenel and Veillet (1951).

RELATION BETWEEN OVARIAN AND MOLTING CYCLES

The proportions of the three molting stages present in each of the four ovarian stages are represented in Figure 1 and Table I. The exclusion of certain stages around molting does not affect, however, the following conclusions, which are also found to be sound statistically by the Chi-square method, using Yate's correction (Table II). (1) In the category of white ovaries, pre- and postmolt crabs predominated, whereas intermolt crabs were rare. (2) As the developmental stages of the ovaries changed through yellow to orange, the proportion of pre- and postmolt crabs decreased, while that of intermolt increased, until at orange the first two were at minimum and the last one was at maximum. At the spent stage the tendency was reversed but the intermolt crabs were still predominant. However, a significant rise in premolt individuals occurred, until at the white stage this, together with the postmolt, was at maximum.

TABLE II

Test of significance by the "Chi-square Method," with Yate's correction, on the relation between ovarian and molting stages in adult female Carcinus. W = white; Y = yellow; O = orange;

Sp = spent. P = probability in per cent. Postmolt includes B₂-C₃; intermolt C₄; premolt D₀-D₂ crabs. Stages D₃-B₁ around molting were excluded in the study (see text).

Change in molting stages	Change In stages of ovaries			
	W to Y	Y to O	O to Sp	Sp to W
Postmolt to intermolt	$P < 1$	$P < 0.1$	$P \neq 5$	$P < 0.1$
Intermolt to premolt	$P < 0.1$	$P < 0.1$	$P < 0.1$	$P \text{ about } 0.1$
Premolt to postmolt		(not tested)		

OVARIAN STAGE AND SHELL COLOR

The more commonly occurring shell colors on the ventral side of the animal are given with the corresponding ovarian stage in Table III and their percentages in Figure 2A. Shell colors are green, yellow and orange-red. In the immature white gonad stage, green was found to be the most frequent shell color. As the ovary became yellow, the frequency of green shell color decreased whereas the

TABLE III

Relation between various ovarian stages and shell color. Per cent in brackets.

Shell color	Ovary				Total
	White	Yellow	Orange	Spent	
Green	73 (57.3)	18½ (34)	35½ (16.7)	6 (5)	133
Yellow	20 (15.7)	14½ (26.6)	33½ (15.8)	7½ (6.3)	75.5
Orange-red	29½ (23.1)	19½ (35.7)	138 (65)	98 (82)	285
Brown	4 (3.1)	0 (0)	2 (0.9)	1½ (1.3)	7.5
Blue	1 (0.8)	2 (3.7)	3½ (1.65)	6½ (5.4)	13
Total	127½ (100)	54½ (100)	212½ (100)	119½ (100)	514

yellow and orange-red increased. At the orange ovarian stage both yellow and green shell frequency decreased while orange-red increased. This latter tendency continued to the spent stage.

Chi-square tests with Yate's correction indicated significance in the above changes (Table IV).

TABLE IV

Test of significance by the "Chi-square Method" with Yate's correction on the relation between ovarian stages and shell color in adult female Carcinus. W = white; Y = yellow; O = orange; Sp = spent.

Change in color on shells	Change in stage of ovaries			
	W → Y	Y → O	O → Sp	Sp → W
Green to orange-red	$P = 5$	$P < 0.1$	$P < 1$	—
Orange-red to green	—	—	—	$P < 1$
Green to yellow	$P < 5$	—	—	—
Yellow to orange-red	—	$P < 0.1$	$P < 1$	—
Orange-red to yellow	—	—	—	$P < 0.1$

We should therefore accept that the change in each of the above-mentioned cases is significant, and arrive at the conclusion that the green shell color, present most abundantly in the white ovarian stage, turns to yellow and orange-red as the crab's ovary becomes fully mature and spent.

Here the yellow appeared to be transitional shell color at the transformation between green and orange-red.

MOLTING AND SHELL COLOR

The numbers of green, orange-red and yellow shell colors included in the three molting stages are included in Table V and their percentages plotted in Figure 2B. Here at postmolt more green than orange-red or yellow was present. At intermolt, orange-red increased sharply while the other two decreased. At premolt, orange-red and yellow decreased while green increased.

TABLE V

Relation between molting stages and shell color. Postmolt = stages B_2-C_3 ; intermolt = stage C_4 ; Premolt = stages D_0-D_2 . Per cent in brackets.

	Postmolt	Intermolt	Premolt	Total
Green	54 (42.4)	55 (17)	24 (37.8)	133
Yellow	36 (28.2)	35 (10.8)	4½ (7.1)	75½
Orange-red	27 (21.1)	226 (70)	32 (50.4)	285
Brown	3 (2.3)	3½ (1.8)	1 (1.6)	7½
Blue	7½ (6)	3½ (1.8)	2 (3.2)	13
Total	127½ (100)	323 (100)	63½ (100)	514

Similar statistical tests are summed up in Table VI.

The contrasting occurrence of green and orange-red is therefore established in the molting as well as maturing cycles. From Figure 2B, it can be seen that most intermolt crabs have orange-red shells. Further (Fig. 1), more intermolt crabs were found with mature (orange) rather than spent ovaries. Yet (Fig. 2A) a smaller proportion of crabs with orange-red were found with mature

TABLE VI

Test of significance by the "Chi-square Method", with Yate's correction, on the relation between molting stages and shell colors in adult female Carcinus crabs. Postmolt includes B_2-C_3 ; intermolt C_4 ; premolt D_0-D_2 crabs.

Change in molting stages	Change in color of shells	
	Green to orange-red	Orange-red to yellow
Postmolt to intermolt	$P < 0.1$	$P < 0.1$
Intermolt to premolt	$P < 1$	P about 70
Premolt to postmolt	P about 1	P about 0.1

(orange) ovaries than spent. This was due to the fact that crabs with orange ovaries included a significantly higher proportion of green and yellow (especially green) shells (Fig. 2A and Table III).

It was noted that crabs smaller than about 35 mm. (not included in this study) carapace width had much less orange-red on the shells than larger ones. They appeared green in color at most stages, though at premolt, additional blue tinges on certain parts of the shell could be distinguished. When newly molted, in the

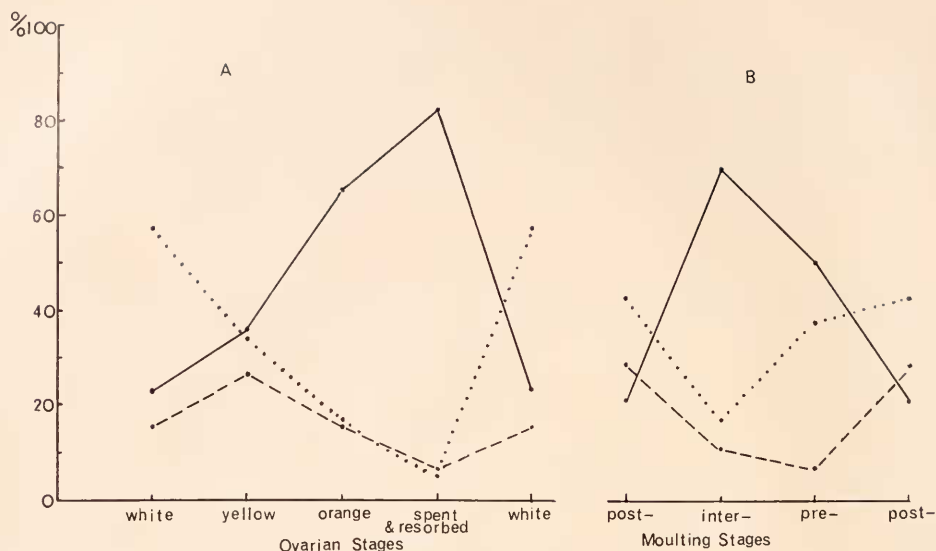


FIGURE 2. A. Ovarian stage and shell color. B. Molting stages and shell color. Post-molt, intermolt and premolt are here defined in the same way as in Figure 1. Green; yellow -----; orange-red ———.

aquarium, they usually acquired bright golden yellow, or less often, brownish color, on the underside. The reason for such different color-changing in the smaller crabs is not yet understood.

DISCUSSION

It was mentioned above that the present method of collection excluded non-feeding crabs around molting time. If, then, those characteristics discussed here did not bias the sampling by trapping, then we may make the following conclusion.

The three cycles, ovarian maturation, molting, and color change, are apparently under different hormonal control in *Carcinus* since any combination of their stages could occur, though correlation between certain stages of the different cycles is found.

Such correlations support, in general, the conclusions of Démeusy and Lenel (1954), and of Démeusy (1963), that the ovarian and molting principles are antagonistic to each other in *Carcinus*, that egg-laying takes place when the crabs are at their intermolt stage, and that molting occurs mostly after the spent stage. The present observations show that, in nature, this antagonism may not always be effective. For example, a molting crab with full-size mature orange ovary was recorded twice during the study.

The color in the shell is undoubtedly the result of biochemical transformations during the metabolism of carotenoids, as indicated by previous workers (Lenel and Veillet, 1951, etc.). These authors have demonstrated that removal of the eyestalks from freshly molted *Carcinus* resulted in a marked reddening, affecting the new cuticle as well as the epidermal chromatophores. According to them,

this reddening is due to the dissociation of the brown and green astaxanthin-protein complexes. This would probably explain the formation of the orange-red color in the present study (see Goodwin, 1960, for review of the subject).

It is difficult to assign any advantage to color changes during the molting and ovarian-developing cycles. However, there is known to be some habitat change during the cycle.

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SUMMARY

1. The three decapod characters, molting, ovarian developing and shell color changing, known to be under hormonal control were studied in the female shore crab, *Carcinus maenas* (L.), by the sampling method.

2. Results indicate that (a) the above three cycles do not appear to be under the same hormonal control; (b) a negative correlation exists between molting and ovarian maturation. This confirms, in general, experimental results of previous authors that there is an antagonism between molting and ovarian maturation. On the other hand, this antagonism does not seem to be always effective in nature. (c) A contrasting occurrence of green and orange-red shell color is established in the molting as well as ovarian maturation cycles. (d) The significance of the shell color changes is discussed.

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